EXAMINATION OF THE EPIDERMIS BY THE STRIP METHOD OF REMOVING HORNY LAYERS

I. Observations on Thickness of the Horny Layer, and on Mitotic Activity after Stripping*

HERMANN PINKUS, M.D.

Some years ago, Wolf (1) developed a new method of examining the surface relief of the skin by stripping off the most superficial horny layer by means of transparent adhesive tape. He demonstrated that a one-layered sheet of horny cells will stick to the tape which then can be mounted on a glass slide and examined at any desired magnification.

It occurred to me that Wolf's method might be valuable in many other ways for gaining more information not only about the horny layer, but about the entire epidermis. Two applications of the strip method are presented in this preliminary communication.

A. Thickness of the Horny Layer

It is not difficult to count the number of layers of the rete mucosum by counting the nuclei in the usual perpendicular sections. But it is almost impossible to count or even to estimate the number of horny layers. It was found that ordinary Scotch Tape ® is well suited for the performance of the strip method. A strip 1.25 cm ($\frac{1}{2}$ inch) wide and about 5 cm long was applied to the flexor surface of the forearm, rubbed lightly to assure adhesion, and then stripped off with a quick movement. For this purpose one end was grasped and the tape was rolled back on itself. It was then applied, sticky side down, to a clean microslide and examined under the microscope. Wolf's observation was borne out. A single sheet of horny cells adhered to the tape, and the various details of surface relief described by him could be studied easily. The sheet of cells was not quite complete, but an estimated two thirds of the skin surface had been stripped off. The surface of the treated skin did not differ appreciably from the surroundings. A second strip of scotch tape was applied and removed and showed a very similar picture under the microscope. The procedure was then repeated over and over with the intention of stripping off as many layers as possible. After four or five applications, the surface looked slightly more dull than the surroundings, but it was not until 24 strips had been applied and pulled off that a definite change was seen. Small shiny reddish areas of irregular size and shape appeared, continued to increase in number with each application, and became coalescent. At the same time, a slight burning sensation was perceived. The cells which adhered to the tape became more single and few in number, but no nuclei or keratohyalin

* From the Department of Dermatology, Wayne University Medical College, Detroit, Mich.

Received for publication February 15, 1951.

granules were seen in the fresh unstained film. After 30 applications, the skin surface appeared almost uniformly reddened, shiny, but dry. The appearance was that of a mild "friction burn." Examination with a magnifying glass showed numerous capillaries shining through in a distribution suggesting the papillary pattern. There was no moisture, and the epidermis proper appeared intact.

It was concluded that probably most of the horny layer had been stripped off, but that the sealing coat which is formed by eleidin and keratohyalin layers above the epidermal lymph spaces had not been broken. A rough estimate is that between one half and two thirds of the area of each tape was covered with cells. This leads to the conclusion that between 15 and 20 layers of horny cells had been present on the treated skin. This was the flexor surface of the forearm of a 45 year old man with slight tendency to keratosis pilaris, but otherwise normal skin.

The same procedure was performed on the corresponding site of a 45 year old woman. 20 strips were obtained, but the test was terminated at a slightly earlier stage of denudation. In a 14 year old boy, 22 strips were necessary for a similar result. Further tests with more subjects and other regions of skin are in progress.

B. Replacement of Stripped Horny Layer

After 12 hours, the skin of the first subject's forearm showed slight redness and slightly perceptible swelling which extended a short distance beyond the stripped area. This had subisded after 24 hours, leaving a slightly krinkled pink surface. At this time, a 2 mm punch biopsy was taken in local anesthesia without previous antiseptic application. Another biopsy was taken after 48 hours. Care was taken to place the biopsy sites 2.5 cm apart in the median line of the strip. The specimens were fixed in formalin, embedded in paraffin and cut into serial sections of 10 μ in order to gain information about the mechanism of replacement of the lost horny layer. While it is generally agreed that the epidermis replaces itself by mitotic division of the basal and prickle cells, objections to this concept have been raised repeatedly because the mitotic index of normal epidermis is so low. Thuringer (2) found figures of 1:2414 in adult scalp, and 1:268 275 in adult ear epidermis. The two sides of the question were presented recently in papers by Hoffman (3) and by Andrew and Andrew (4). It is well known that in those skin diseases which like psoriasis and eczematous dermatitis lead to increased shedding of the horny layer numerous mitoses usually are present in the epidermis. It was thought interesting to see what effect simple removal of most of the horny layer would have on normal epidermis.

In the 24 hour specimen, the surface was covered in part by a single granular layer and one or several layers of normal horn cells, in part by several layers of flat nucleated parakeratotic cells without granular layer. Stretches of one type alternated with the other. It was evident that the stripping process had been of uneven efficacy. The rete mucosum consisted of 4 to 6 layers of cells. The most striking feature was that in many places the basal cells were greatly elongated, their nuclei and the supranuclear cap of pigment granules far removed from the base. The basal layer often constituted almost half of the thickness of the entire

epidermis. Nuclei of prickle cells varied in size considerably, and there was a number of binucleate cells. Under oil immersion, the nuclei of basal cells and prickle cells below the granular layer, or where that was absent, below the obviously pyknotic layer, were counted in seven consecutive sections. Individual sections contained between 1164 and 1785 (average 1483) resting nuclei for a total of 10381. A total of 24 nuclei in mitosis were seen. The mitotic index was 1:433.

The 48 hour specimen showed a continuous granular layer, usually 3–4 rows thick. Above it, there were either anuclear or parakeratotic layers. The rete mucosum had 4–6 layers, the basal layer consisted mostly of cuboid cells, with a few long ones in between. In many places it was obvious that the lowest row of cells contained no pigment while the next higher layer showed the supranuclear melanin cap usually present in the basal cells. Cell count in ten consecutive sections showed an average of 1076 (881–1392) resting nuclei below the granular layer. There were 165 mitoses among the total of 10922 nuclei. The mitotic index was 1:66. Binucleate cells were rare.

It is obvious from these findings that the gentle removal of most of the horny layer is sufficient to stimulate a veritable burst of mitotic activity in the epidermis. The duration of mitosis has been variously estimated as from 15 minutes to two to three hours. The best estimate probably is one half hour, even according to Lewis and Lewis (5) who gave the highest figure. These authors included in their two to three hour estimate much time for prophasis and for reconstruction of the nucleus. I purposely counted only nuclei from the stage of late prophase through telophase. If we accept half an hour as the average time of mitosis a mitotic index of 1:66 means that cell number may double in a 33 hour period. It is of course impossible to say if the very peak of mitotic activity was observed 48 hours after the insult, and how long that activity continued.

No definite instances of direct nuclear division (amitosis) were seen, but the presence of a fair number of binucleate cells in the 24 hour specimen indicates that amitosis may play a role in the early effort of the epidermis to maintain its integrity. Other mechanisms of this effort apparently are hasty incomplete keratinization (parakeratosis) and increase of cell size, particularly in the basal layer. The most important part in the restitution of the lost cells however is played by mitotic division of the basal and prickle cell layer of the epidermis. The mitotic figures often occurred in groups, and most of them were in or close to the basal layer. A more detailed report will be given after more material has been collected.

SUMMARY

Wolf's strip method of removing the most superficial horn cells for microscopic study of surface relief was adapted to the layer-for-layer removal of most of the stratum corneum.

It is estimated that the stratum corneum on the flexor surface of the forearm is from 10 to 20 layers thick.

The epidermis replaces the lost horny layer mainly by mitotic division of basal

and lower prickle cells. Mitotic indices of 1:433 and 1:66 were found 24 and 48 hours after stripping.

REFERENCES

1. Wolf, J.: Das Oberflächenrelief der menschlichen Haut. Zeitschr. f. mikr.-anat. Forschung **47:** 351–400, 1940.
2. Thuringer, J. M.: Studies on cell division in the human epidermis. Anat. Record **40:** 1–13, 1928.
3. Hoffman, J. G.: Quantitative analysis of the growth of epidermis. Arch. Pathol. **47:** 37–43, (Jan.) 1949.
4. Andrew, W. and Andrew, N. V.: Lymphocytes in the normal epidermis of the rat and of man. Anat. Record. **104:** 217–241, 1949.
5. Lewis, W. H. and Lewis, M. R.: The duration of the various phases of mitosis in the mesenchyme cells of tissue cultures. Anat. Record **13:** 359–368, 1917.